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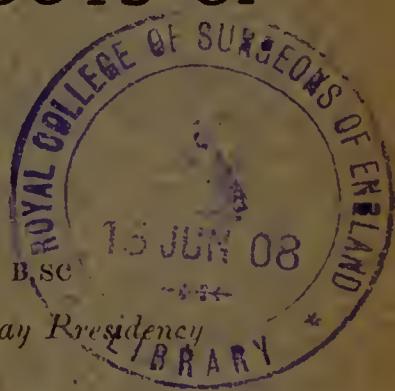
Memoirs of the Department of Agriculture in India

NOTE ON A TOXIC SUBSTANCE EX- CRETED BY THE ROOTS OF PLANTS

BY

F. FLETCHER, M.A., B.Sc.

Deputy Director of Agriculture, Bombay Presidency



AGRICULTURAL RESEARCH INSTITUTE, PUSA

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NOTE ON A TOXIC SUBSTANCE EXCRETED BY THE ROOTS OF PLANTS.

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IT has more than once been suggested that plants, like animals, excrete (from their roots) material that is no longer of use to them or that are bye-products of the process of metabolism, and that such substances are injurious to the kinds of vegetation by which they are excreted. Brugmans was apparently the first to suggest this, and it has, at various times, been affirmed by Plenk, Humboldt, Cotta, De Candolle and others, denied by Hedwig, Braconnot, Walser, Boussingault, Unger, Meyen and others and has for long been considered as non-existent except with regard to carbon dioxide and possibly an acid phosphate and formates.

Again, Dr. Gyde (Trans-Highland and Agricultural Society, 1845-47, pp. 273-92) in water cultures obtained on evaporating the residual liquid, a very small amount of yellowish or brown substance, a portion of which was organic in character. He concluded that the amount excreted was very small, and that the substance was not injurious to the plants that gave rise to it.

From observations on crops growing in the field, the writer some years ago (while in Egypt) came to the conclusion that certain phenomena could only be explained on the theory of excretion. This was especially the case with cotton crops in which a grass was allowed to grow as a weed. The cotton, grown under irrigation, did not revive on application of more water; its poor state was therefore not due to lack of moisture in the soil. Manures likewise seemed to have comparatively little effect towards improvement; appropriation by the weed of food materials was therefore not the cause of the poor growth. Aeration had as little effect as manure.

Observations in the field were resumed in India, and these tended to very materially strengthen the view that materials injurious to other crops were excreted by the roots of certain common crops in India; this was especially the case with sorghum. The system of mixed crops very prevalent in the unirrigated tracts in India gave full opportunity for a number of observations to be taken, and on the strength of these, experiment was resumed both in the field and in water culture.

FIELD EXPERIMENTS.

The results of field experiments which commenced (in India) in the season 1903-04 were unreliable in the two following years owing to the failure of the monsoon. In the season of 1906-07, the rains were more nearly normal, and the following observations were made on the Surat Experiment Station; they agree in kind and differ only in degree from those obtained in the previous year under a short rainfall.

This Station is on typical deep black cotton soil and receives an annual rainfall of about 42 inches, all falling between the middle of June and the end of September.

The soil is very retentive of moisture as will be obvious from the fact that cotton sown in June survives until the end of March, though no rain is received during the last six months previous to the final picking of the crop. The composition of this soil, as kindly analysed by Dr. Leather, is as follows:—

MECHANICAL COMPOSITION.

Fine Gravel, 1 mm.	2.2
Sand, 1 mm. -0.2	3.4
," 0.2—0.05 mm.	42.2
," 0.05—0.01 mm.	22.8
," 0.01 mm.	20.8
					91.4
Stones	3.6
Fine earth, 2 mm.	96.4
					100.0

CHEMICAL COMPOSITION.

Insoluble silicates and sand	68.06
Ferrie oxide	8.83
Alumina	11.07
Lime	2.79
Magnesia54
Potash42
Soda31
Phosphoric acid09
Sulphuric acid06
Carbonic acid94
Chlorine07
Organic matter and combined water	6.82
					100.00
Total nitrogen036
Nitric nitrogen00014
Available phosphoric acid016
Available potash012

The observations were taken as follows :—

Plots of various crops were grown side by side, each plot being sown by means of a drill in such a way that the rows of crops (2 ft. apart) were parallel in all the plots. Further, several plots were left fallow as it was found that some crops growing on the border of a fallow yielded at a rate as much as 10 times as great as the rate in the centre of the plot (Annual Report of the Bombay Farms for 1904-05). Again, cotton and sorghum were sown in alternate rows in the same plot.

The following observations were then made :—

- (1) The yield of the row of each crop bordering on fallow.
- (2) The yield of the row of each crop bordering on a plot bearing another crop.
- (3) The yield of a row of each crop in the centre of a plot bearing only that crop.
- (4) The yield of a row of cotton when grown with a row of sorghum of each side (at a distance of 2 ft.).
- (5) The yield of the row of sorghum when grown with a row of cotton on each side (at a distance of 2 ft.).

In all cases the results given are the mean of a large number of observations.

Observation (1) is the nearest approach possible under the conditions of the experiment, to the yield of a crop when grown isolated, *i.e.*, influenced neither by plants of the same nor of other species. In the present season it is hoped to obtain a nearer approximation.

The results obtained (1906-07) are given in the table following, the total yield per acre (dry weight) of the crops being given in lbs. :—

TABLE I.

CROPS (of which yield is given.)	YIELD (fruit and vegetative portion) in lbs. per acre of the crops in first column when grown bordering on a plot of :				
	Fallow.	Sorghum.	Cajanus.	Cotton.	Sesamum.
Sorghum ...	10,735	4,830	8,051	8,802	8,158
Cajanus ...	4,633	694	1,621	1,621	2,409
Cotton ...	3,817	229	763	1,145	1,259
Sesamum ...	1,650	<i>Nil</i>	198	247	643

Taking the yield next to the fallow as a rough approximation to the yield of the isolated crop, we get the following percentage reduction in these yields produced by a neighbouring plot* of another crop :

TABLE II.

CROPS (of which percentage decrease is given).	Percentage DECREASE in yields of crops in first column when grown near			
	Sorghum.	Cajanus.	Cotton.	Sesamum.
Sorghum ...	55	25	18	24†
Cajanus ...	85	65	65	48
Cotton ...	94	80	70	67
Sesamum ...	100	88	85	61‡

* In the present season observations will be made on the yield of crops when a row is bordered on *both* sides by another crop.

† Since sesamum is extremely sensitive to the presence in its vicinity of other plants whether of the same or other species, the yield next to fallow (Table II) is probably lower than the *isolated* yield by a much greater extent than in the case of all other crops, since the latter are less sensitive. The figures in the bottom line of Table II should therefore all be increased.

‡ This is the result of one observation only and is probably too high since sesamum will not grow at all within 2ft. (the width of the rows) of sorghum.

It is to be noted that pending further and more precise experiment the figures in Table II are to be considered as indicating only the order of the influence of various plants on one another and that too only under the particular conditions of soil and climate under which the experiment was conducted. On lighter soils and under a more evenly distributed rainfall the percentage reductions are apparently less. With this reservation and since the decreased yields are not restored by either irrigation, manure, aeration or light,* it appears legitimate to draw the following conclusions :—

- (1) All plants excrete substances which are toxic both to themselves and to other species.
- (2) The quantity of material excreted by the different crops varies when reckoned per unit area of a field sown in the ordinary way.
- (3) The sensitiveness of crops to the same quantity of the excreted substance varies with the variety of the crop.
- (4) The substance excreted by all crops is apparently identical.

The last statement is made under a further reservation pending the isolation in a pure state and analysis of the excreted substance or substances. There is, however, nothing in the facts so far observed either in the field or in water culture inconsistent with the identity of the substance excreted by all plants. On the other hand, if the substance excreted varied with the species, we should not expect the regularity found in Table II. Thus, reading that table vertically, the order of sensitiveness to a given amount of the substance excreted by sorghum appears to be (beginning with the least sensitive) :—sorghum, cajanus, cotton, sesamum. The same order holds good for the same plants towards the substance excreted by cajanus, by cotton and by sesamum. If now the excrement from sorghum differed not only in quantity but also in kind from that of cajanus, we should

* All these factors have been proved by experiment to be incapable of correcting except very partially the poorer growth except in the case of certain manurial substances (*see* later).

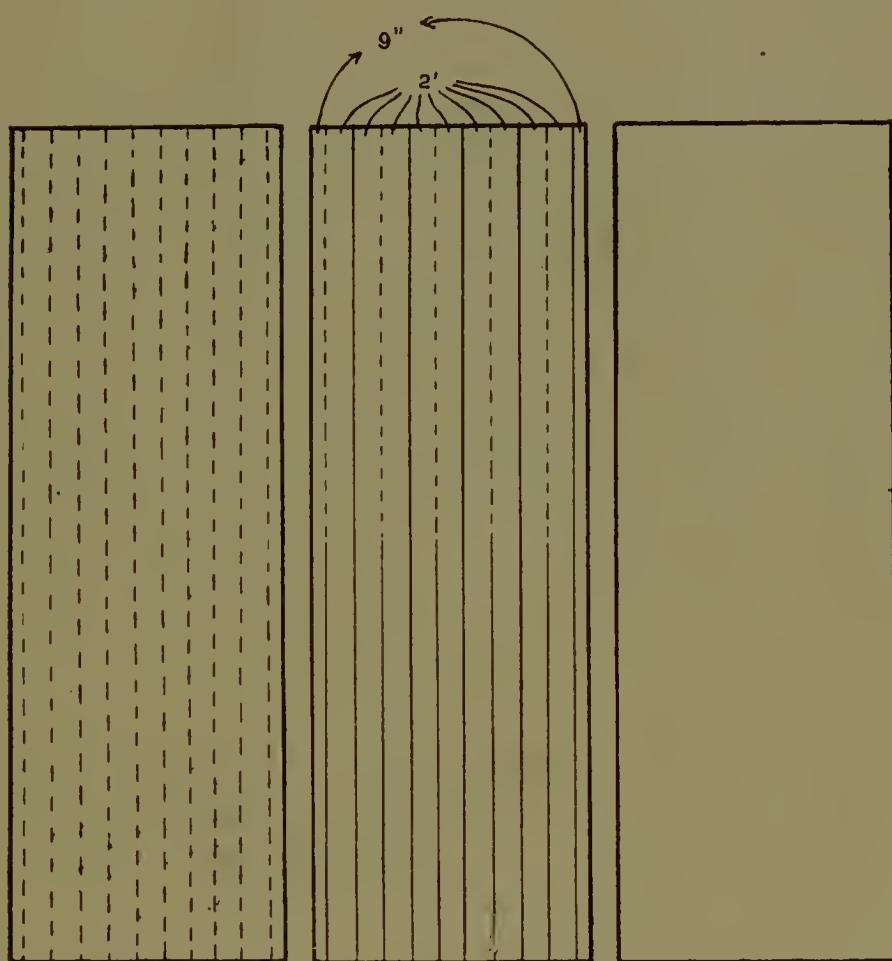
expect that if in two species of plants one was the more resistant to a given quantity of sorghum excrement, yet the other might be the more resistant to the excrement from, say, cajanu. This has, however, been observed to be the case neither in the field nor in water cultures.

Further, the amount of substance excreted (per unit area of a field sown under the conditions of the experiment) by the various crops *appears* to be in the same order. For, reading the columns of Table II horizontally, we find that sorghum excretes an amount of substance which reduces its own yield by 55 per cent., while cajanu excretes an amount that reduces the yield of sorghum by 25 per cent., cotton an amount that reduces the yield of sorghum by 18 per cent. The regularity of the table as read thus horizontally may, however, be deceptive.

On the Dharwar Experimental Station a few observations (on other crops) were made, which indicate that gram (cicer) is about equally sensitive and equally toxic with wheat, that both are equally sensitive with sesamum but less toxic than the latter, while linseed is similar to cotton in both respects except perhaps that it is more sensitive.

In the experiment where cotton and sorghum were grown in alternate rows (2 feet apart) the following results given below in Table III were obtained. The figures are so remarkable that they are given in full. The experiment was made on $\frac{1}{4}$ -acre plots, each 191 yards long and $6\frac{1}{2}$ yards wide, of which the record is known since 1897-98, when my predecessor, Mr. Mollison, laid them out for an excellent series of rotation and manure experiments. In numbering the rows, the numbers of the rows go in the same direction as the numbers of the plots themselves, so that the first row of a plot borders on the plot whose number is next lower and at a distance of $3\frac{1}{2}$ feet from it. The relative positions of the different crops will be obvious from the accompanying plan of one of the plots in which dotted lines represent rows of cotton; continuous thin lines, rows of sorghum; and continuous thick lines, the border of the plot.

PLATE I.



PLAN SHOWING RELATIVE POSITIONS OF DIFFERENT CROPS AT DHARWAR
EXPERIMENTAL STATION.



The treatment of the plots whose northern halves were thus sown with alternate rows of cotton and sorghum had been since 1897-98 (except 1905-06) as follows:—

TABLE III.

Number of plot.	Treatment.
1 & 2 ...	Sown in alternate years with cotton and sorghum, 5 tons of farm-yard manure being applied before every cotton crop.
11 ...	In a three-year rotation <i>Crotalaria</i> ploughed in as green manure (followed in the same year by sesamum and cajanus) preceded cotton and this sorghum, each cotton crop receiving 5 tons of farm yard manure.
14 ...	Similar to plot 11, but the green manure was not followed by a crop in the same year.
24 & 25 ...	Sown in alternate years with cotton and sorghum, no manure being given.

In the season 1906-07 all the plots in the original series were sown with cotton, and all those in the duplicate series with sorghum except in both series the northern halves of plots 1, 2, 11, 14, 24, 25 (which were sown as indicated above) and plots 12 and 15 which were left fallow. The average yield (in lbs.) per acre (total produce) of the separate half rows is given in the following table:—

TABLE IV.

Number of plot.	SERIES I.			SERIES II. (DUPLICATE OF SERIES I).		
	SOUTHERN HALF.	NORTHERN HALF.		SOUTHERN HALF.	NORTHERN HALF.	
		Cotton.	Sorghum.		Cotton.	Sorghum.
1 ...	1,260	543	6,342	3,392	144	9,078
2 ...	1,464	499	6,017	3,397	99	9,811
11 ...	3,332	2,286	11,103	6,220	1,749	2,297
14 ...	3,187	2,595	11,760	6,091	1,572	8,550
24* ...	896	385	3,550	2,752	151	6,089
25* ...	776	278	3,960	2,791	69	5,897

* Reckoned on $\frac{1}{3}$ ths of the plot only that was beyond the influence of a neighbouring fallow area.

At first sight the extraordinary difference between the yields (northern halves only) of series I and its duplicate series II would seem to indicate that the soil differed in the two

cases. The whole difference is, however, due to last year's cropping, series I being then under sorghum and series II under cotton except plots 11 and 14 which were fallow in both series. This accounts for the great difference in the yields of the northern halves of plots 1 and 2, 24 and 25, sorghum yielding much better after cotton (series II) than after sorghum (series I). The converse might appear to be the case with cotton which in series II (after cotton) has yielded less than in series I (after sorghum), and the whole might appear to be an example of the benefit of rotation. The small yield of cotton in series II is, however, due in part at least to the more vigorous growth of the sorghum with which it was sown as a mixed crop and only to a small extent to the fact that the preceding crop was cotton.

WATER CULTURES.

A large number of experiments in water culture were started some years ago. It is unnecessary to give the preliminary investigations on this point. The greater part of the results obtained by me have been brought together for a final test during the last few months, and these only will be here recorded.

Preparation of Solution of Excreta.

In December last, final water cultures were started on the Dharwar Experimental Station in a number (in all 20) of earthenware dishes (12 inches in diameter and 4 inches inside depth). In each of these dishes 4 litres of well water was placed, and over this a circular teak board perforated by 90 holes, $\frac{1}{4}$ inch in diameter, was supported by strings, to which were attached counterpoise weights hanging over the outside of the dish. Seeds were germinated in crushed quartz, and when the radicle was about an inch long, were transferred to the water culture dishes; a radicle was passed through each hole in the board and held in place by a small wad of cotton. In each 4 litres of water therefore 90 seedlings were planted; those that failed were replaced by others.

The crops were harvested, roots and all, every 21 days, and in all, three crops were taken from each dish between the 10th of January 1907 and May 15th. The water in the dishes was kept up to 4 litres by adding well water every few days.

The crops grown were cotton, sorghum, cajanus indicus, sesamum, wheat, gram (*Cicer arietinum*).

The air dry weights (including the roots) of the three harvests of the several crops is given in Table V.

TABLE V.

Air dry weights of crops and the amount of water into which the excreta had passed.

No.	Name of crop.	No. of plants grown.	Air dry weight in grammes.	Volume of well water in which their excreta was finally contained.	Total volume of water evaporated and replaced.	
					Litres.	Litres.
1	Cotton ...	246	9.170	2.3		19.00
2	Sorghum ...	224	6.026	2.3		18.25
3	Cajanus ..	157	6.407	2.3		19.25
4	Sesamum ...	133	0.746	1.2		18.75
5	Wheat ...	261	7.766	1.2		19.75
6	Gram ...	261	19.308	1.2		27.75

The volume of water remaining in the dishes on harvesting the third crop was allowed to stand in a room until its volume was reduced to the quantity stated, this quantity having been indicated in previous tests to be the best for the final test.

Water culture in the excretory solution.

For brevity the various solutions obtained will be called 'cotton water,' 'sorghum water,' etc.

The water cultures were made in wide-mouthed bottles holding 100 c. c. when filled up to the neck. The mouth was plugged with a teakwood stopper in which four holes ($\frac{1}{4}$ inch diameter) were bored. Through these holes the radicle or the plumule of seedlings germinated in crushed quartz was passed, the seedlings being supported in place by a small wad of cotton.

In the case of cotton, cajanus and sesamum, the root was passed downwards through the stopper; in the other cases the

plumule was passed upwards. In the latter cases the plant was adjusted so that the seed was in position just above the surface of the water in the bottle.

The seedlings were very carefully chosen from several hundreds grown in crushed quartz, so as to be as nearly equal among themselves as possible. The state at which the seedlings are best suited to the purpose in hand was found by repeated experiment to be as follows :—

- (1) *Cotton* when the spread of the cotyledons is $1\frac{1}{2}$ inches.
- (2) *Sorghum* when the first and second leaves are equal (both about 1 inch long).
- (3) *Cajanus* when the 'spread' of the first leaves is 4 inches.
- (4) *Sesamum* as soon as the cotyledons have assumed a horizontal position.
- (5) *Wheat* as for sorghum.
- (6) *Gram* when the first three leaves have expanded.

The strength of the solutions had been so arranged (by allowing to evaporate) that no plant (of the size indicated) would grow in any of them for more than about ten days. The time between transplanting into the bottles and the times of commencement of wilting and of complete drying up were carefully recorded. The bottles were also weighed every morning and in some cases several times a day to find the amount of transpiration, this amount having been proved (Bulletin No. 28, Bureau of Soils, U. S. A.) to be a measure of the increase of the plant in dry weight.

In all cases two, and in some cases as many as six bottles were treated in the same way; both as regards the solution they contained and the crops grown in them. It was found that with careful selection of seedlings of each size the difference between duplicates either in time of withering or in loss by transpiration was extremely small. The observations here recorded are in all cases the mean of the total number of bottles sown in the particular manner indicated. They are set forth in the following tabular statement :—

Progress of crops named in Column I when grown in water containing muller exerted by crops in Columns 2 to 7 and in distilled water.

CROPS THAT HAD PREVIOUSLY GROWN IN THE WATER.									DISTILLED WATER.	
	Sorghum.	Cajanus.	Cotton.	Sesamum.	Wheat.	Gram.	Period of growth.	Weight in grams of transpiration.		
1	2	3	4	5	6	7	8	9		
Sorghum	1 0 5 3 * 15·5 0 *	1 0 3 all 12·2 10 0 8	1 0 3 all 10·5 10 1 5·7	1 0 2 all 3·2 8 all 3	1 0 2 all 3·0 10 all 3	1 0 2 all 3·0 5 all 3	1 0 2 all 3·0 5 all 3	1 0 2 all 3·0 5 all 3	6 2 all 10 5 all 10	4·7
Cajanus	18								
Cotton	12·5								
Sesamum	6·0								
Wheat	7·0								
Gram	17·7								

(R)P.S. (q) which progress is indicated.

• Plants did not wither at all.

† Though the cotton did not wither, it ceased to transpire after the 3rd day.

The data given in the table prove that all the plants tried, wither in the same order in the different solutions; thus all do worst in the "gram water," "sesamum water" being the next most toxic, followed in order by wheat, cotton, cajanu, sorghum.

This order could of course be easily changed by diluting any one or more of the solutions, the strengths of which in the experiment are quite arbitrary.

The fact of this regularity appears to favour the view put forward above that the substances excreted by various plants are identical, and that the solutions used differ only in concentration and not in kind.

Nature of the Toxic Substance.

It was at first thought that the toxic matter might be an albumose or similar substance. The solutions, however, all gave negative results, on the application of the biuret and other tests for these compounds.

The fact that tannic acid precipitated and corrected the toxic material suggested the presence of an alkaloid.

It is interesting to note that leaves containing tannic acid are systematically used as manure in the spice gardens and rice fields of Canara* and that the cultivators' opinion as to the manurial value of the leaves of any particular variety of tree corresponds apparently to the amount of tannic acid contained in the leaf. Thus in the order of preference the leaves of the following trees (among others) are utilized in this way:—

Hirda (*Terminalia chebula*).

Matti (Do. *tomentosa*).

Honal (Do. *paniculata*).

Kanagal (*Dillenia pentagyna*).

That it is not the ash constituents of these leaves that produce the manurial effect is obvious from the fact that if the leaves be burnt and the ashes applied to pepper—one of the spices to

* Mollison—"Cultivation of Betel, Palm, Cardamom and Pepper in the Kanara District of the Bombay Presidency" (Bulletin No. 20 of the Department of Land Records and Agriculture, Bombay, 1900).

which the leaf manure is applied—the pepper vine is killed. Similarly neither irrigation nor farm-yard manure serves the purpose of the leaves; the latter therefore serves neither for storage and regulation of water nor as a supply of nitrogen.

It was indeed these facts that first suggested to me the possibility of the toxic substance being either an albuminous substance or an alkaloid for both of which tannic acid acts as a precipitant.

A preliminary examination of the solutions only has been as yet made, but this appears to prove that it is an alkaloid that is excreted by all the plants experimented with, and further that the substance is identical in all cases.

The solutions examined consisted of both well and distilled water in which plants had grown.

The principal reactions obtained are as follows:—

Phosphomolybdic acid	A white precipitate.
Phosphotungstic acid	Do. do.
Mayer's Reagent	Do. do.
Tannic acid	Do. do.
Platinum chloride	Precipitates on standing.
Iodised potassium iodide	
Mercuric chloride	A coagulated white precipitate.

The substance is thrown down in concentrated solutions only as a white flocculent precipitate on adding caustic potash. A similar precipitate is thrown down immediately on adding potassium nitrate, potassium chloride, potassium sulphate or sodium chloride and after some time on adding sodium nitrate or sulphate. Potassium chloride and sulphate and sodium chloride produce coagulated precipitates and apparently precipitate the substance most completely of the reagents tried. The precipitate is insoluble in water, alcohol and all the usual organic solvents, but soluble in acids and alkalies. This precipitate can be titrated with an acid using methyl orange as indicator. It is therefore apparently the base itself and not a salt. The salt formed on titration is acid to litmus as is also distilled water in which plants have grown. This fact apparently accounts for statements that free acids or acid salts are exuded by plant roots. Further, on precipitation the solution becomes distinctly acid to methyl orange.

Ammonium sulphate and dilute sulphuric acid also cause precipitation (of the sulphate?) of the substance after some time; so also do sodium acetate, ammonium (but not sodium) phosphate, ammonium molybdate (with nitric acid), ferric chloride (soluble in acetic acid).

With greater concentration doubtless other substances than those here indicated would cause precipitation.

The substance cannot be separated out by shaking its ammoniated solution with amyl alcohol, chloroform, or either, hot or cold. It is also insoluble in alcohol.

The solution produces after a few minutes a blue precipitate (in a green solution) with a mixture of ferric chloride and potassium ferricyanide. It also decomposes potassium permanganate in the cold with production of a stable red precipitate (of the permanganate?) destroyed by boiling or excess of the reagent. Reduction also takes place with Fehling's solution and with silver oxide in ammonia.

It is easily decomposed by heating at 100°C when in the solid state.

The solid dissolves in strong acids and Frohde's reagent without colour, except in the case of nitric acid which gives a yellow solution. Potassium bichromate after strong sulphuric acid gives a green colour, changing to blue.

The above reactions were given by all the solutions named in Table V and appear to indicate the identity of the toxic substance in the case of all plants.

The substance is present in the solution in combination with citric acid. I could find no trace of phosphate or formate as stated to exist by Czapek, though the original solution before elimination of the base, simulates many of the reactions of these salts.

Without having reference to the current literature on the subject it is impossible to compare the reactions above given with those obtained with any of the known alkaloids. Its marked insolubility appears to differentiate it from all the commoner alkaloids except pseudomorphine and rhæodine with which it is improbable that it is identical.

The amount of substance given out by the roots is not inconsiderable. For instance, the precipitate obtained by adding potassium sulphate to a solution containing the excrement of 10 cotton plants growing until their combined air-dry weight was 4 grammes, weighed, when dry, 21 grammes.

Sesamum in its early stages of growth, appears actually to excrete a greater amount of material than it builds up in its own substance.

CONCLUSION.

The bearing of the phenomena described in this article on the question of rotation of crops is obvious.

The question may, however, be put why cotton, for instance, which grows so feebly *near* sorghum (Table IV) grows at least as well if not better, *after* sorghum than after cotton. From experiments now in progress it appears that this is explicable as follows:—

When cotton is growing near sorghum the roots of the latter exude the toxic substance into the soil in large quantities. This spreads rapidly through the soil into the subsoil especially during the rainy season, and neighbouring cotton plants are not protected by the fact that their tap roots go down far below the zone in which the sorghum roots are situated. When cotton follows sorghum, however, the condition of affairs is different; the toxic substance remaining, at the time of harvesting, in the roots of the previous sorghum crop is now being given out slowly in the course of the decay of those roots,* and is held entangled in the organic matter of the roots, largely in the zone of soil in which the roots of sorghum spread. Each crop thus fouls the soil for a crop of the same variety, whose roots will take the same course as the previous crop, more than for a crop whose roots spread in another layer of the soil.

The precipitation of the toxic substance by most of the mineral manures in common use indicates the manner in which many manures act in increasing crop yields.

* That the roots of sorghum and other crops exert an extraordinarily toxic effect when mixed with soil in which plants are then grown has been proved by the writer in a set of pot experiments.

While this note has been going through the press I have received Bulletin No. 40 of the Bureau of Soils, (United States of America) by Messrs. Schreiner and Reed on "Some factors influencing soil fertility." In this Bulletin the authors come to the conclusion that "the excreta of the cow pea roots are very slightly toxic to roots of wheat seedlings" (page 35), and that "the excreta of oats are more toxic to the roots of wheat seedlings than those of corn or cow peas—a conclusion that is substantiated by the results obtained in crop rotations" (page 36).

The experimental data given in the Bulletin do not justify these conclusions but only indicate that the excreta from cow peas *when in the arbitrary concentration obtaining in their experiments* are very slightly toxic to roots of wheat seedlings *when these latter are at the stage of growth of those used in the experiment.*

I find that very young plants are not affected by a toxic solution of given strength so rapidly as older plants, doubtless because the latter, owing to more rapid transpiration, take in the toxic substance in larger quantities.

Again, the impossibility of the statement made with regard to the excreta of oats being more toxic than that of cow peas or corn is self-evident, implying as it does that any quantity *however great* from oats is more toxic than any quantity *however small* from cow peas or corn.

The media that were compared contained quite arbitrary amounts of excreta from an arbitrary number of plants growing for an arbitrary period, it being stated that "the agar containing their excretions was obtained in each case by planting a large number of seedlings in a dish of soft agar and allowing them to grow for eight to fifteen days according to the kind of plant employed."

There are no data in the Bulletin under reference which indicate that the excreta from all the plants tried are not identical in character.